REMARKS/ARGUMENTS:

Reconsideration of the above identified application is respectfully requested.

In the Office action dated April 27, 2006, claims 1, 3-5, and 7-16 are pending and rejected. Claims 1, 3-5, and 7-16 are rejected under 35 U.S.C. § 103 over CN 1346648 (hereinafter "CN '648").

Applicant respectfully traverses the rejections as set forth below. In addition, Applicant has amended claims 7 and 11 to correct minor errors. No new matter has been introduced.

Claim Rejection Under 35 U.S.C. § 103(a)

Claims 1, 3-5, and 7-16 are rejected under 35 U.S.C. § 103(a) as being unpatentable over the CN '648 reference. There is no dispute that the CN '648 reference was published on May 1, 2002, which is later than the claimed priority date of the Chinese counterpart application, which has a filing date of April 21, 2002.

The Examiner cited 37 CFR § 1.55 to support her reason for not allowing Applicant to rely upon his Chinese foreign priority date to disqualify the CN '648 reference as prior art, which, according to the Examiner, is because Applicant has not provided a translation of the foreign priority document.

In response to the rejections, Applicant hereby attaching an English translation of the Chinese priority document and a certificate of translation as Exhibit 1, in support of her traversal of the rejections.

Applicant respectfully requests the withdrawal of the rejections in view of the English translation of the Chinese priority document.

In view of the foregoing, the rejections have been overcome and the claims are in condition for allowance, early notice of which is requested. Should the application not be passed for issuance, the examiner is requested to contact the applicant's attorney to resolve the problem.

Respectfully submitted,

Date: June 26, 2006

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CERTIFICATE OF VERIFICATION OF TRANSLATION

I, Gesheng Huang of Zhongzi Law Office,
am the translator of the attached Chinese application No.02109532.9, which is the claimed priority of PCT/CN03/00290 and I state that the following is a true translation to the best of my knowledge and belief.

Signature .....Gesheng Huang.....
Name Ge sheng Huang

Dated this ....15.....day of .....June.....2006.

An Injection made from Ixeris Sonchifolia Hance for Treatment of Cardio-Cerebral Vascular Diseases and Fundus Diseases and Method of Producing Thereof

Technical Field

The present invention relates to an injection made from Chinese herb and method of producing thereof, and more particularly to a lyophilized powder of *Ixeris Sonchifolia Hance* for injection and method for producing thereof, for treatment of cardio-cerebral vascular diseases and fundus diseases.

Background Art

Currently, Chinese herbal injections for treatment of cardio-cerebral vascular diseases and fundus diseases include *Ixeris Sonchifolia Hance* injection, which for clinical application is a brown-yellow transparent liquid derived from *Ixeris Sonchifolia Hance* of *Compositae* and extracted as intravenous injection starting from full plants. The main pharmaceutical components of the intravenous injection are flavone and adenosine. The coexistence of phyto-flavone and -adenosine in *Ixeris Sonchifolia Hance* has shown a remarkable complementary effect for treatment of cardio-cerebral diseases, which has already been demonstrated by pharmacodynamics study and clinical application. The pharmacological effects of *Ixeris Sonchifolia Hance* injection consist in its efficacies of: 1. increasing coronary artery flow, lowering cardiovascular resistance, resisting myocardial infarction, enhancing collateral circulations, reducing myocardial oxygen consumption and improving cardio-microcirculations, which are useful for the treatment of coronary heart diseases, angina pectoris, chest distress and breath shortness, and myocardial infarction; 2. reducing platelet conglomeration, increasing the activity of fibrinolysin, inhibiting thrombosis, decreasing the viscosity of blood plasma and serum, increasing the electrophoresis velocity of erythrocytes,

lowering cerebro-vascular resistance, increasing cerebral blood capacity and promoting restoration of neural function, which are useful in the treatment of cerebral infarction (cerebral thrombosis); 3. improving microcirculation disorders caused by bacteria, systemic microcirculation disorders caused by polymer dextran, and fundus microcirculation, and dilating fundus artery, which are useful in the treatment of fundus diseases, such as central retinitis, optic atrophy and retinitis pigmentosa. However, *Ixeris Sonchifolia Hance* injections exhibit poor stability and can only stand short-term storage. The contents of flavone and adenosine have been determined by High Performance Liquid chromatography (HPLC) and derivatization method, which show that adenosine content reduces from 15.0 μg/ml prior to finishing of the aqueous injection to 6.7 μg/ml thereafter, while for flavone, from 0.25 mg/ml to 0.169 mg/ml, with a lose of 55.3% and 32.4% of the prescribed, respectively. Six months later, adenosine content reduces to 6.5 μg/ml, while flavone content is 0.133 mg/ml. This can also be verified by subjecting *Ixeris Sonchifolia Hance* aqueous injection wherein flavone and adenosine contents are respectively 5.07 mg/ml and 24.37 μg/ml, to ten-day accelerated stress test carried out at 80℃ in an oven, followed by content determination of both, in doing so, merely 3.16 mg/ml and 12.18 μg/ml are left for flavone and adenosine, respectively (Tab.1). In view of the considerable lose of both active ingredients of flavone and adenosine during preparation and storage, the therapeutic effect of the injection is seriously impacted. Moreover, *Ixeris Sonchifolia Hance* injection exhibits a distinct change in color of solution. The absorbance measured at 400 nm using ultraviolet spectrophotometer during preparation of the aqueous injection from stock extract, ranges from 0.338 prior to sterilization to 0.423 thereafter, and further increases to 0.443 after 6 months with darkened color. It should be further noted that HPLC spectra of the injection exhibit poor similarity, that is to say, there exist major differences among HPLC spectra of different batches of aqueous injections yet formulated from the same stock extract. The stability behaviour of same kind of

commercial aqueous injections by random sampling (Batch No. 20000303, 20000503, 20010120) shows that: flavone contents are 0.27 mg/ml, 0.51 mg/ml and 0.30 mg/ml, while for adenosine, the contents are 1.11 μg/ml, 0.147 μg/ml and 0.00 μg/ml, respectively

In a conclusion, *Ixeris Sonchifolia Hance* aqueous injection is susceptible to various factors such as preparation, storage, and thus is difficult to be controlled in quality, which greatly expenses the therapeutic effect of *Ixeris Sonchifolia Hance* injection.

Contents of the Invention

The object of the present invention is to provide a Chinese herbal injection for treatment of cardio-cerebral vascular diseases and fundus diseases, and method of producing thereof, which not only overcome the defects existing with *Ixeris Sonchifolia Hance* aqueous injection, but allow stable and readily controlled quality, little lose of flavone and adenosine contents, therefore ensure a more safe and effective clinical use, and be of great advantage for storage.

The object of the invention is achieved by a Chinese herbal injection for treatment of cardio-cerebral vascular diseases and fundus diseases, characterized in that it is in a form of lyophilized powder of *Ixeris Sonchifolia Hance* for injection, wherein the content ratio of flavone to adenosine is 5 mg：15 μg or 5 mg：30 μg.

The method of producing the Chinese herbal injection according to the invention is effected as following: clean *Ixeris Sonchifolia Hance* is added to 25~30 times amount of water for 3 hours decoction, strained, micro-strained and concentrated until 1ml of concentrate corresponds to 0.5g of crude herb; the concentrated decocting solution is then cooled to below 40℃, and 10% calcium oxide emulsion is added under stirring to adjust pH to 10～11, filtered, and the precipitate is weighed; said precipitate is suspended in 5.3 times of

ethanol, 25% strength of sulfuric acid solution is added to adjust pH to 3～4, followed by through stirring and filtration; 40% sodium hydroxide solution is added to the filtrate and adjusted pH to 7～7.5, filtered, and ethanol is then recovered therefrom and eliminated by evaporation, water for injection is added to allow 1ml as corresponding to 4g of crude herb; then refrigerated below -8℃ for 12 hours, filtrated; boiled for 15 minutes by adding 0.1～0.2% active carbon, and allowed to stand at -5℃ for more than 24 hours, filtrated, adjusted to pH 7.0～7.5, then filtered through cardboard, sintered funnel and microporous membrane (pore diameter of 0.45 μm), sealed after filling, and sterilized(115℃, 30min), to provide the extract; to said extract is added stabilizing agents or subsequently supporting agents, and stirred to allow complete dissolution, further treated by adding active carbon for injection, and filtrated, the resulting transparent non-pyrogen solution is then charged in vials or ampoules, pre-frozen at -40～-60℃ for 1～3 hours, vacuumed (vacuum degree of 1～20Pa) by suction, and dried at elevating temperature for 20～40 hours to the final of 25～40℃. Lyophilized powder of *Ixeris Sonchifolia Hance* for injection is thus obtained.

The benefits of the invention consist in that: by formulating *Ixeris Sonchifolia Hance* containing flavone and adenosine into lyophilized powder of *Ixeris Sonchifolia Hance* for injection, the defects of unstable quality and loss of flavone and adenosine going with said *Ixeris Sonchifolia Hance* aqueous injection are overcome, in addition, easy control of quality, little lose of flavone and adenosine contents have been achieved, therefore ensuring a more safe and effective clinical use, and be of great advantage for storage.

Specific Embodiments

Example 1:

1kg of clean *Ixeris Sonchifolia Hance* are put into a decocting pot, to which 25～30 times amount of water are poured over for 3 hours decoction. The decocting mixture is strained, microstrained, and then transferred to a

concentrator for concentration until 1 ml of the concentrate corresponds to 0.5g of crude herb. The concentrated solution is then allowed to cool to 39℃, stirred and added with 10% calcium oxide emulsion to adjust pH to 10. After filtration, the precipitate formed is taken for weighing, then suspended in an amount of ethanol 5.3 times by weight of the precipitate itself, and 25% strength of sulfuric acid solution is further added thereto to adjust pH to 3. The suspension is well stirred, filtered, and 40% sodium hydroxide solution is then added to the filtrate to bring pH to 7. After filtration, ethanol is recovered from the filtrate and subsequently eliminated by evaporation. Water for injection is then added until 1ml corresponds to 4g of crude herb, which is then refrigerated at -9℃ for 12 hours, filtered, boiled for 15 minutes by adding 0.1% active carbon, and further allowed to stand at -5℃ for 24 hours. The solution is further filtered, adjusted to pH 7, then filtered through cardboard, sintered funnel and microporous membrane (pore diameter of 0.45 μm), sealed after filling, and sterilized (115℃, 30min), to provide the extract. To the extract obtained is added 0.05% EDTA or sodium citrate as stabilizing agent, and stirred to allow complete dissolution, further treated by adding 0.1% active carbon for injection, and filtered. The resulting transparent non-pyrogen solution is then charged in each vial or ampoule of 2ml, pre-frozen at -40℃ for 3 hours, vacuumed by suction (vacuum degree of 15 Pa), and dried at elevating temperature for 20 hours to the final of 25℃. Lyophilized powder of *Ixeris Sonchifolia Hance* for injection thus is prepared.

Example 2

1kg of clean *Ixeris Sonchifolia Hance* are put into a decocting pot, to which 25～30 times amount of water are poured over for 3 hours decoction. The decocting mixture is strained, microstrained, and then transferred to a concentrator for concentration until 1 ml of the concentrate corresponds to 0.5g of crude herb. The concentrated solution is then allowed to cool to 35℃, stirred and added with 10% calcium oxide emulsion to adjust pH to 10. After

filtration, the precipitate formed is taken for weighing, then suspended in an amount of ethanol 5.3 times by weight of the precipitate itself, and 25% strength of sulfuric acid solution is further added thereto to adjust pH to 3.5. The suspension is well stirred, filtered, and 40% sodium hydroxide solution is then added to the filtrate to bring pH to 7.3. After filtration, ethanol is recovered from the filtrate and subsequently eliminated by evaporation. Water for injection is then added until 1ml corresponds to 4g of crude herb, which is then refrigerated at -10℃ for 12 hours, filtered, boiled for 15 minutes by adding 0.12% active carbon, and further allowed to stand at -5℃ for 25 hours. The solution is further filtered, adjusted to pH 7.2, then filtered through cardboard, sintered funnel and microporous membrane (pore diameter of 0.45 μm), sealed after filling, and sterilized (115℃, 30min), to provide the extract. To the extract obtained are added 0.1% sodium bisulfite or sodium pyrosulfite or mixture thereof as stabilizing agent and 3% mannitol as supporting agent, stirred to allow complete dissolution, further treated by adding 0.05% active carbon for injection, and filtered. The resulting transparent non-pyrogen solution is then charged in each vial or ampoule of 3ml, pre-frozen at -45℃ for 2.5 hours, vacuumed by suction (vacuum degree of 18 Pa), and dried at elevating temperature for 30 hours to the final of 30℃. Lyophilized powder of *Ixeris Sonchifolia Hance* for injection thus is prepared.

Example 3

1kg of clean *Ixeris Sonchifolia Hance* are put into a decocting pot, to which 25～30 times amount of water are poured over for 3 hours decoction. The decocting mixture is strained, microstrained, and then transferred to a concentrator for concentration until 1 ml of the concentrate corresponds to 0.5g of crude herb. The concentrated solution is then allowed to cool to 20℃, stirred and added with 10% calcium oxide emulsion to adjust pH to 10.5. After settling for 12 hours, the precipitate is filtered off and weighed, then suspended in an amount of ethanol 5.3 times by weight of the precipitate itself,

and 25% strength of sulfuric acid solution is further added thereto to adjust pH to 4. The suspension is well stirred, filtered, and 40% sodium hydroxide solution is then added to the filtrate to bring pH to 7. After filtration, ethanol is recovered from the filtrate and subsequently eliminated by evaporation. Water for injection is then added until 1ml corresponds to 4g of crude herb, which is then refrigerated at -11℃ for 12 hours, filtered, boiled for 15 minutes by adding 0.15% active carbon, and further allowed to stand at -5℃ for 24 hours. The solution is further filtered, adjusted to pH 7.4, then filtered through cardboard, sintered funnel and microporous membrane (pore diameter of 0.45 μm), sealed after filling, and sterilized (115℃, 30min), to provide the extract. To the extract obtained are added 0.05% sodium sulfite or ascorbic acid or mixture thereof as stabilizing agent, or gassed with nitrogen, and then 3% dextran as supporting agent, stirred to allow complete dissolution, further treated by adding 0.05% active carbon for injection, and filtered. The resulting transparent non-pyrogen solution is then charged in each vial or ampoule of 1ml, pre-frozen at -50℃ for 2 hours, vacuumed by suction (vacuum degree of 10 Pa), and dried at elevating temperature for 25 hours to the final of 35℃. Lyophilized powder of *Ixeris Sonchifolia Hance* for injection thus is prepared.

Example 4

1kg of clean *Ixeris Sonchifolia Hance* are put into a decocting pot, to which 25～30 times amount of water are poured over for 3 hours decoction. The decocting mixture is strained, microstrained, and then transferred to a concentrator for concentration until 1 ml of the concentrate corresponds to 0.5g of crude herb. The concentrated solution is then allowed to cool to 10℃, stirred and added with 10% calcium oxide emulsion to adjust pH to 11. After filtration, the precipitate is taken for weighing, then suspended in an amount of ethanol 5.3 times by weight of the precipitate itself, and 25% strength of sulfuric acid solution is further added thereto to adjust pH to 3. The suspension is well stirred, filtered, and 40% sodium hydroxide solution is then

added to the filtrate to bring pH to 7. After filtration, ethanol is recovered from the filtrate and subsequently eliminated by evaporation. Water for injection is then added until 1ml corresponds to 4g of crude herb, which is then refrigerated at -12℃ for 12 hours, filtered, boiled for 15 minutes by adding 0.2% active carbon, and further allowed to stand at –5℃ for 24 hours. The solution is further filtered, adjusted to pH 7.5, then filtered through cardboard, sintered funnel and microporous membrane (pore diameter of 0.45 μm), sealed after filling, and sterilized (115℃, 30min), to provide the extract. To the extract obtained are added 0.02% sodium thiosulfate as stabilizing agent, and 5% lactose or glucose or mixture thereof as supporting agent, stirred to allow complete dissolution, further treated by adding 0.01% active carbon for injection, and filtered. The resulting transparent non-pyrogen solution is then charged in each vial or ampoule of 4ml or 5ml, pre-frozen at -60℃ for 1 hour, vacuumed by suction (vacuum degree of 20 Pa), and dried at elevating temperature for 40 hours to the final of 40℃. Lyophilized powder of *Ixeris Sonchifolia Hance* for injection thus is prepared.

The stabilities under high temperature of flavone content and adenosine content in the lyophilized powder for injection and aqueous injection are compared in Tab.1.

No.	Dosage form	Flavone (mg/ml) (pre-test)	Flavone (mg/ml) (post-test)	Adenosine (μg/ml) (pre-test)	Adenosine (μg/ml) (post-test)	Appearance
1	Lyophililized powder for injection	5.07	5.03	24.37	23.88	Yellow-brown after dissolving in water
2	Aqueous injection	5.07	3.16	24.37	12.18	Dark brown-yellow

Lyophilized powder of *Ixeris Sonchifolia Hance* for injection is formulated as an injection solution containing 5.07 mg/ml flavone and 24.37 μg/ml adenosine, which is subjected to 10-day accelerated stress test at 80℃ in an oven, then both contents are determined. The contents of flavone and adenosine almost remain unchanged as 5.03 mg/ml and 23.88 μg/ml, while for the aqueous injection, said contents have reduced to 62.33% and 49.99% of the original values.

During clinical application, depending on the requirement of individual condition, different specifications of Lyophilized powder of *Ixeris Sonchifolia Hance* for injection can be dissolved in water for injection, and further added to 250ml sodium chloride or glucose injection for intravenous infusion.

Claims

1. A Chinese herbal injection for treatment of cardio-cerebral diseases and fundus diseases, characterized in that said injection is in a form of lyophilized powder of *Ixeris Sonchifolia Hance* for injection, wherein the content ratio of flavone to adenosine is 5 mg : 15 μg or 15 mg : 30 μg.
2. A method for producing the Chinese herbal injection according to claim 1, characterized in that: clean *Ixeris Sonchifolia Hance* is added to 25~30 times amount of water for 3 hours decoction, strained, micro-strained and concentrated until 1ml of concentrate corresponds to 0.5g of crude herb; the concentrated decocting solution is then cooled to below 40℃, and 10% calcium oxide emulsion is added under stirring to adjust pH to 10～11, filtered, and the precipitate is weighed; said precipitate is suspended in 5.3 times of ethanol, 25% strength of sulfuric acid solution is added to adjust pH to 3～4, followed by through stirring and filtration; 40% sodium hydroxide solution is added to the filtrate and adjusted pH to 7～7.5, filtered, ethanol is then recovered from the filtrate and eliminated by evaporation, and water for injection is added to allow 1ml as corresponding to 4g of crude herb; then refrigerated below -8℃ for 12 hours, filtrated; boiled for 15 minutes by adding 0.1～0.2% active carbon, and allowed to stand at -5℃ for more than 24 hours, filtrated, adjusted to pH 7.0～7.5, then filtered through cardboard, sintered funnel and microporous membrane (pore diameter of 0.45 μm), sealed after filling, and sterilized(115℃, 30min), to provide the extract; to said extract is added stabilizing agents or subsequently supporting agents, and stirred to allow complete dissolution, further treated by adding active carbon for injection, and filtrated, the resulting transparent non-pyrogen solution is then charged in vials or ampoules, pre-frozen at -40～-60℃ for 1～3 hours, vacuumed by suction (vacuum degree of 1～20Pa), and finally

dried at elevating temperature for 20～40 hours to the final of 25～40℃, thus to provide a lyophilized powder of *Ixeris Sonchifolia Hance* for injection.

3. A method as claimed in claim 2, characterized in that said stabilizing agent is EDTA, citric acid (sodium citrate), sodium bisulfite, sodium sulfite, sodium pyrosulfite, sodium thiosulfate, ascorbic acid or nitrogen.
4. A method as claimed in claim 2, characterized in that said supporting agent is mannitol, dextran, lactose or glucose.
5. A method as claimed in claim 2 or 3, characterized in that one or a mixture of more than two kinds of the stabilizing agents is added.
6. A method as claimed in claim 2 or 4, characterized in that one or a mixture of more than two kinds of the supporting agents is added.